

Charge Reversal in Anionic Liposomes: Experimental Demonstration and Molecular Origin

Alberto Martín-Molina,¹ César Rodríguez-Beas,¹ and Jordi Faraudo^{2,*}

¹*Grupo de Física de Fluidos y Biocoloides, Dept. de Física Aplicada, Universidad de Granada, 18071 Granada, Spain*

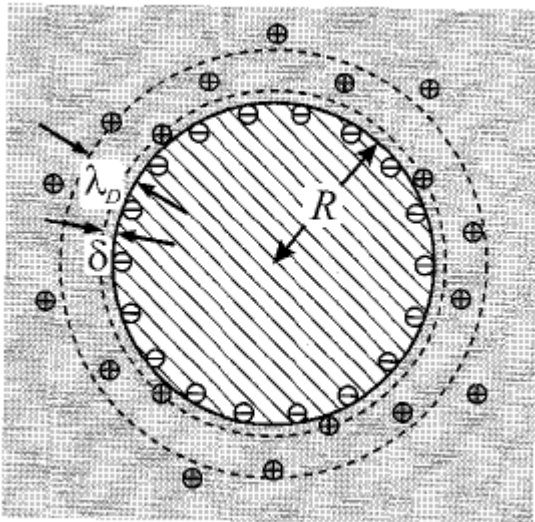
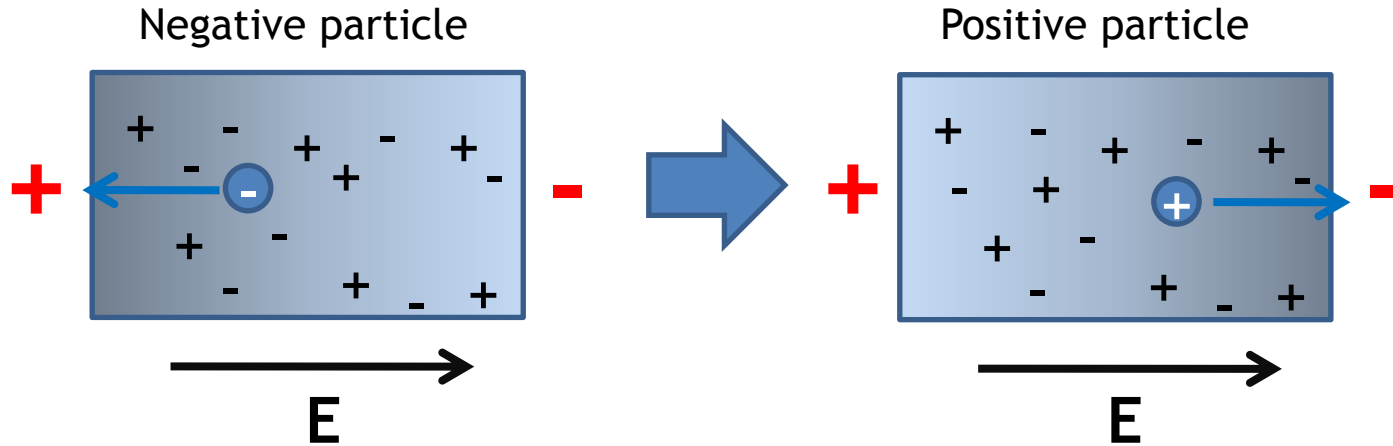
²*Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus de la UAB, E-08193 Bellaterra, Spain*

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We present experimental and simulation evidence for a new mechanism of charge reversal operating only for ions capable to penetrate into soft interfaces. It is based on the preferential solvation of counterions by amphiphilic molecules and hydration water rather than by bulk water. This mechanism does not require high surface charge densities and it is not affected by the addition of 1:1 salt. This behavior is opposite to that observed in systems as diverse as microfluidic channels or latex colloids. The robustness of the mechanism to physiological amounts of 1:1 salt suggests a significant impact in processes involving ion-amphiphile interaction in salty water (typical, e.g., of biophysics).

Electrophoresis - the motion of charged particle

If Charge inversion at colloid particle surface

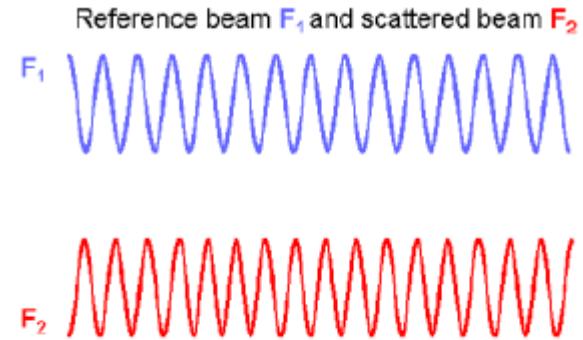
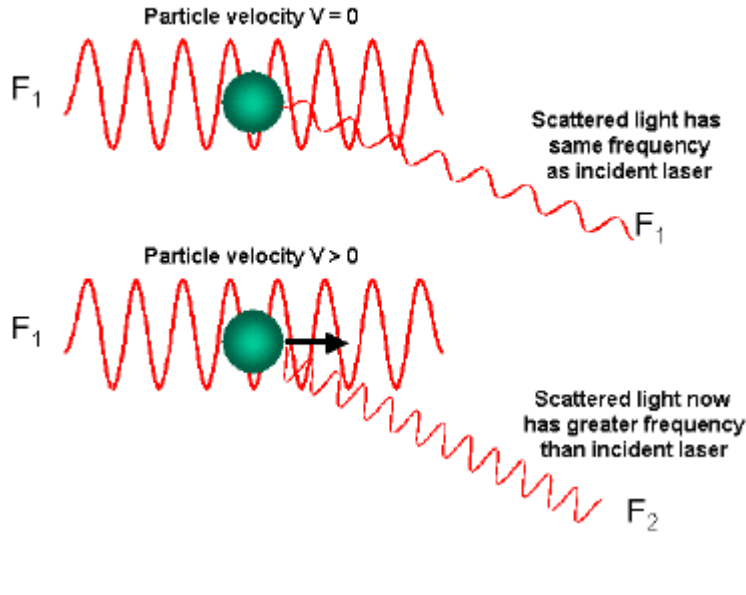


δ : shear plane (slipping plane), λ_D : Debye length

Electrophoretic mobility :

$$v = \mu_e E \longleftarrow \mu_e = \frac{\varepsilon \varepsilon_0 \zeta}{\eta}$$

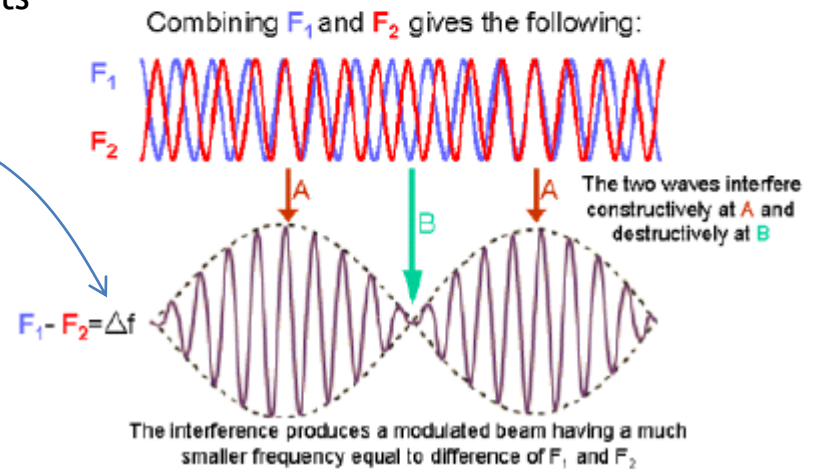
Electrophoresis - Conventional Laser Doppler Electrophoresis



Doppler electrophoresis measures small frequency shifts in scattered light

$$\Delta f \cdot 2\pi = q\mu_e E$$

$$q = (4\pi n / \lambda_0) \sin(\theta / 2)$$



Electrophoresis - Conventional Laser Doppler Electrophoresis

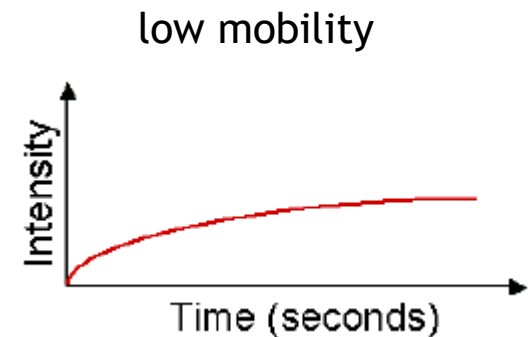
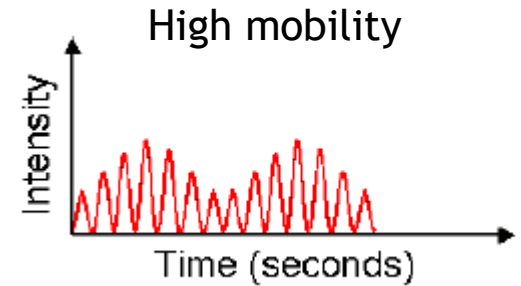
Values of mobility range of $\pm 7 \times 10^{-8} \text{ m}^2\text{V}^{-1}\text{s}^{-1}$ and zeta potential in the range of $\pm 90 \text{ mV}$

In conventional instrument, applied field order 1000 Vm^{-1}

→ velocities of the order $10\text{-}100 \text{ }\mu\text{m/s}$

Table 1. Mobility Ratio for Particles with the Same Zeta Potential in Various Media

Liquid	Viscosity η (cP)	Dielectric Constant ϵ/ϵ_0	Mobility Ratio μ_L/μ_w
Water	00.89	78.0	1.000
Methanol	00.54	33.0	0.700
Toluene	00.56	02.4	0.050
Ethylene glycol	17.00	40.0	0.030
Glycerol	01.20	43.0	0.400
Oleic acid	26.00	02.5	0.001
<i>n</i> -Octane	00.54	02.0	0.040
1:4 Dioxane	01.26	02.2	0.020



Electrophoresis - Phase Analysis Scattering (PALS)

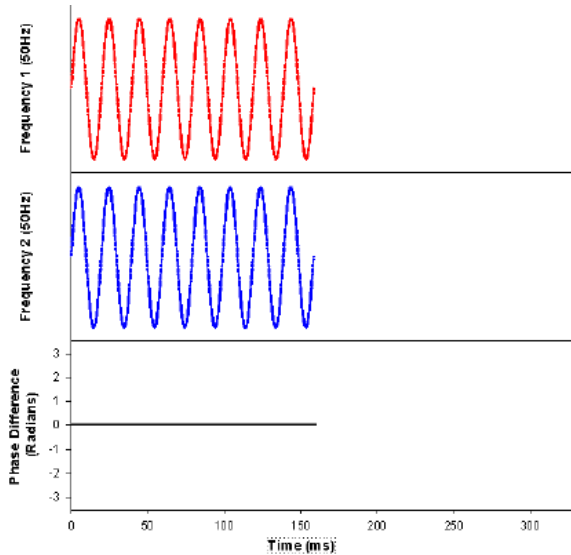


Figure 3: Schematic diagram showing the phase difference between two signals with the same frequency (50Hz)

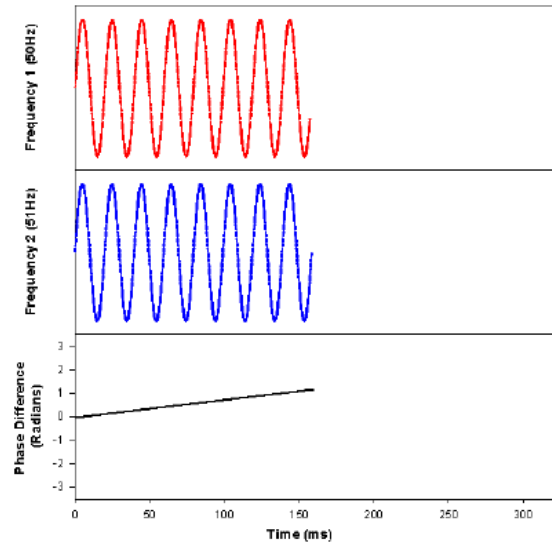


Figure 4: Schematic diagram showing the phase difference between two signals of different frequencies (50 and 51Hz)

Time derivation of the phase :

$$\frac{d\Phi_s(t)}{dt} = \Delta f \cdot 2\pi = \omega_s = q\mu E = q \cdot (v_e \pm v_c)$$

$$\frac{d\Phi_s(t)}{dt} = q \cdot [\langle \mu_e \rangle E(t) \pm v_c]$$

Scattered light signal :

$$S(t) = A \exp[-i(\omega_0 t + \Phi_s)] \quad (1)$$

reference light signal :

$$S_{ref}(t) = \exp(i\omega_0 t) \quad (2)$$

Multiplying (1) and (2) :

$$A \exp[-i(\omega_0 t + \Phi_s)] \exp(i\omega_0 t) = A \exp(i\Phi_s)$$

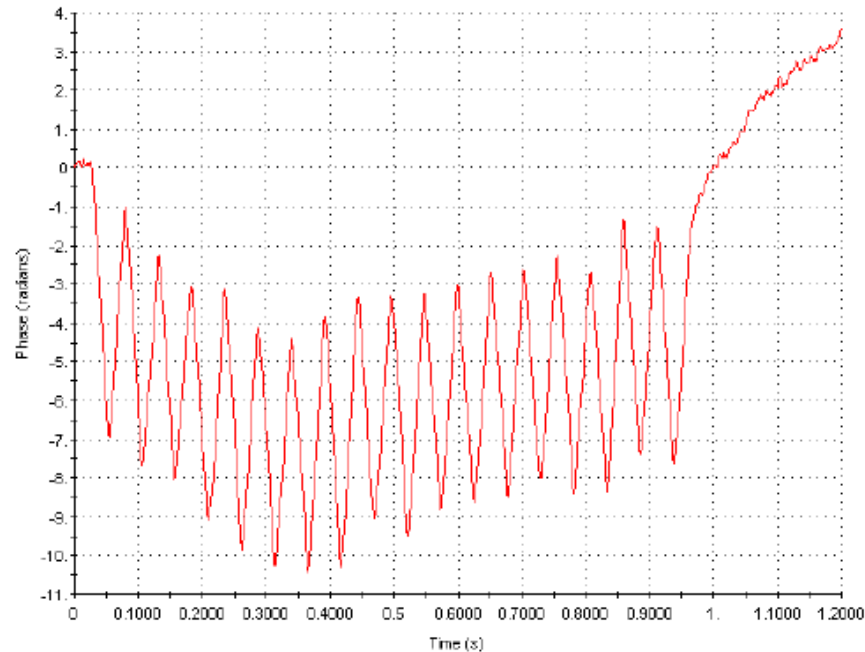
Electrophoresis - Phase Analysis Scattering (PALS)

Phase comparison takes place over many cycles of the applied field

$$\Delta\Phi_s = \Phi_{te} - \Phi_o = \langle A \rangle q \left\{ \left[\int_0^{te} \langle \mu_e \rangle E(t) \pm v_c \right] dt \right\}$$

For a sinusoidal applied field with frequency $\nu = \omega_e/2\pi$

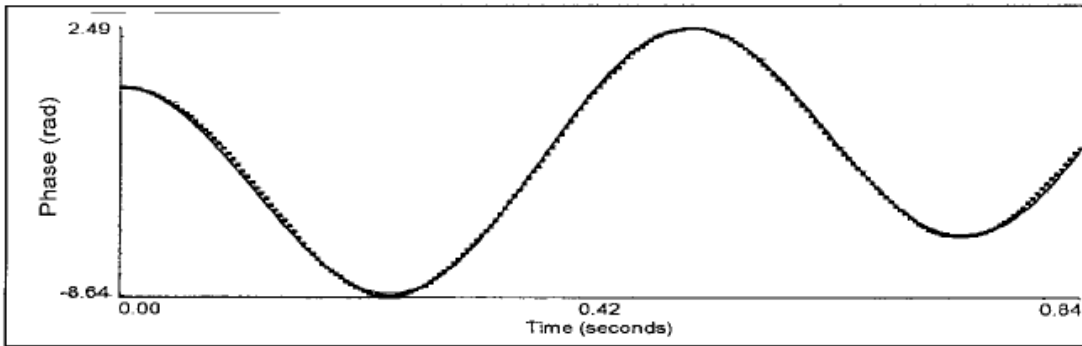
$$\Delta\Phi_s = \langle A \rangle q \left\{ \left[\langle \mu_e \rangle E_0 \cos(\omega_e t_e) / \omega_e \right] \pm v_c t_e \right\}$$



Electrophoresis - Phase Analysis Scattering (PALS)

Measurement Parameters:			
Mean Zeta Potential	= -13.70 mV	Liquid	= Aqueous
Zeta Potential Model	= Smoluchowski	Temperature	= 25.0 °C
Mean Mobility	= -1.07 (μ /s) / (V/cm)	Viscosity	= 0.890 cP
pH	= 9.30	Refractive Index	= 1.330
Conductance	= 1077 μ S	Dielectric Constant	= 78.54
Concentration	= 0.00 mg/ml	Particle Size	= 1.0 nm

Instrument Parameters:			
Sample Count Rate	= 382 kcps	Voltage	= 10.00 V
Ref. Count Rate	= 1288 kcps	Electric Field	= 27.04 V/cm
Wavelength	= 678.0 nm	User1	= 0.00
Field Frequency	= 2.00 Hz	User2	= 0.00

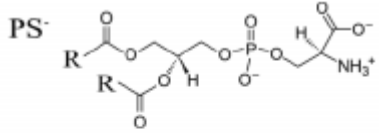


Run	Mobility	Zeta Potential (mV)	Rel. Residual
1	-1.06	-13.58	0.0119
2	-1.04	-13.31	0.0137
3	-1.11	-14.22	0.010
4	-1.12	-14.25	0.008
5	-1.03	-13.	0.0193
6	-1.15	-14.76	0.0083
7	-1.24	-15.86	0.0141
8	-1.16	-14.80	0.0124
9	-0.97	-12.38	0.0106
10	-0.83	-10.56	0.0206
Mean	-1.07	-13.70	0.0131
Std. Error	0.04	0.47	0.0013
Combined	-1.07	-13.70	0.0080

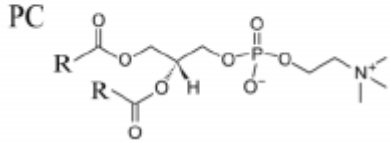
Fig. 4. Raw water and 0.075-mg/l KMnO₄. Autotracking not applied. The dotted curve represents measured data points. The photon count intensities were measured in kilocounts/s (kcps).

Experimental

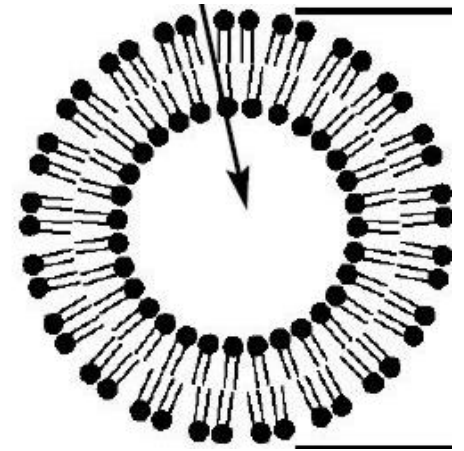
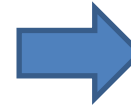
Liposome samples :



Anionic phosphatidiserine (PS⁻)



Zwitterionic phosphatidylcoline (PC)



ZetaPALS - Zeta Potential Analyzer Utilizing Phase Analysis Light Scattering



The electrophoretic mobility of the liposomes at 25 °C was measured as a function of La(NO₃)₃ concentration both in absence of background electrolyte and with 100 mM of added NaNO₃

Results - Electrophoretic mobility

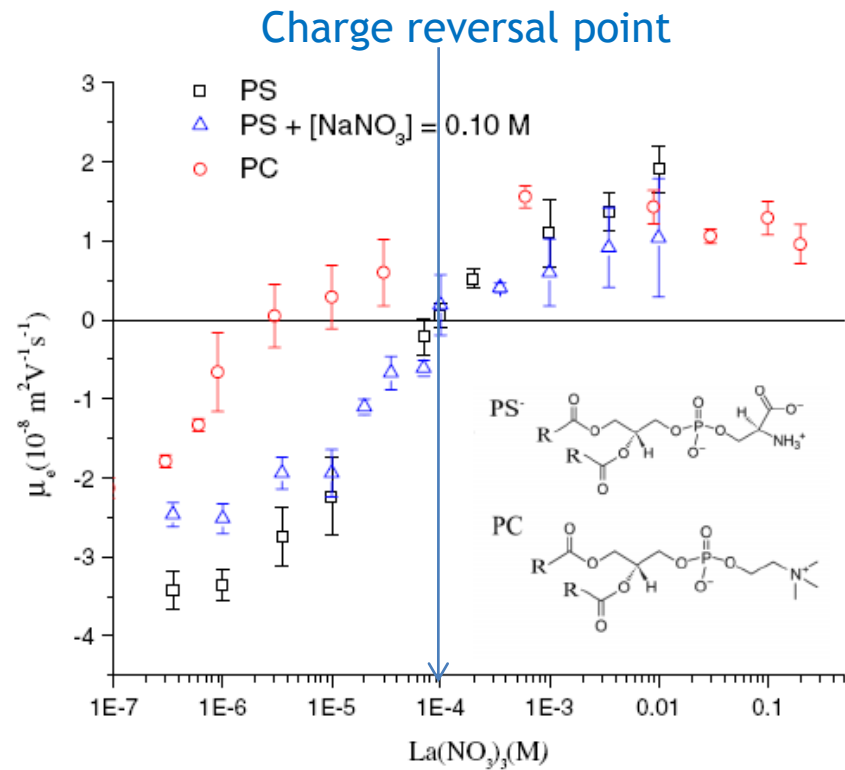


FIG. 1 (color online). Electrophoretic mobility μ_e of PS⁻ and PC liposomes as a function of [La(NO₃)₃]. Squares correspond to PS⁻ (no background electrolyte), triangles to PS⁻ with 100 mM of NaNO₃ as a background electrolyte and circles correspond to PC (no background electrolyte). Inset: Chemical structures of the PS⁻ and PC lipids.

The competition for binding between different ions is controlled by the respective free energies of interaction.

For La³⁺

$$\Delta\mu = k_B T \ln[c_0 \delta z a_p q_c / e] \approx -9k_B T$$

c_0 : counterion concentration
 (10⁻⁴ M = 6 × 10⁻³ ions/nm³)

δz : layer thickness (1 nm)

a_p : mean area per phospholipid (55,4 Å²)

q_c counterion charge (+3e)

For Na⁺

$$\mu \approx -3.5k_B T \quad \text{In other reference}$$

For PC

$$\approx -12k_B T \quad \text{In other reference}$$

Results - Molecular Dynamics simulation (MD) [DLPOLY2]

Distribution of electronegative atoms

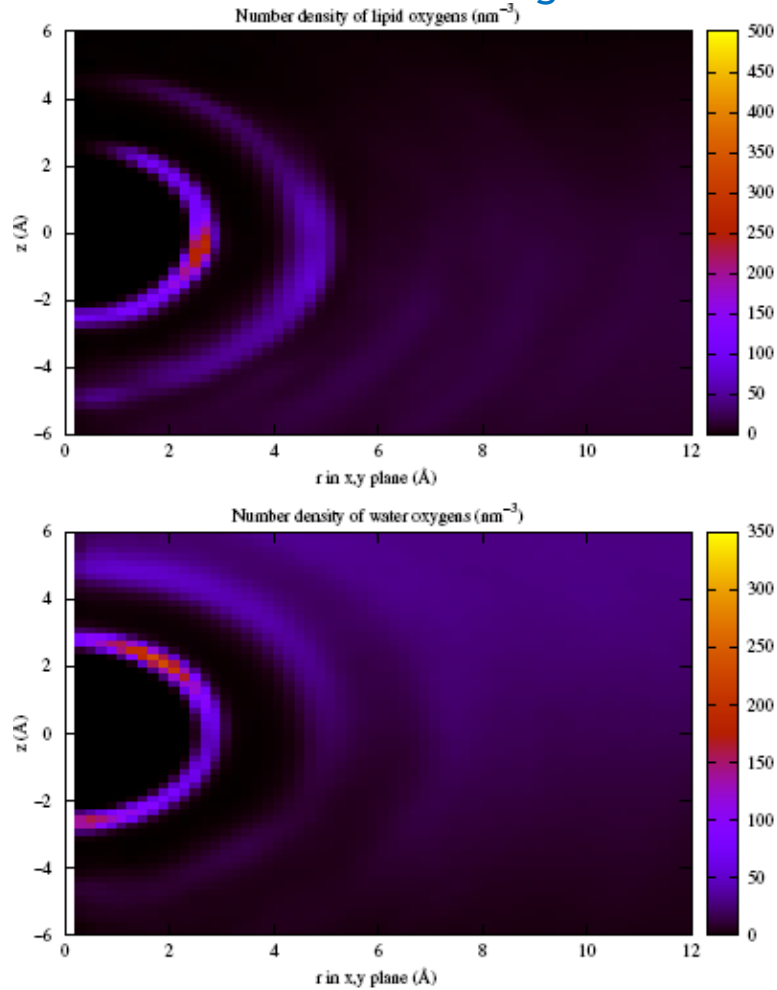


FIG. 2 (color online). Structure of the interface near adsorbed La^{3+} counterions from MD simulations. (a) Particle density (atoms/ nm^3) of oxygen atoms from PS^- molecules around adsorbed La^{3+} cations. The cylindrical coordinates r, z centered at the adsorbed ions are defined so that z is negative towards the membrane interior and positive towards the bulk water; (b) Same as (a) but for oxygen atoms from water molecules.

Results - Molecular Dynamics simulation (MD) [DLPOLY2]

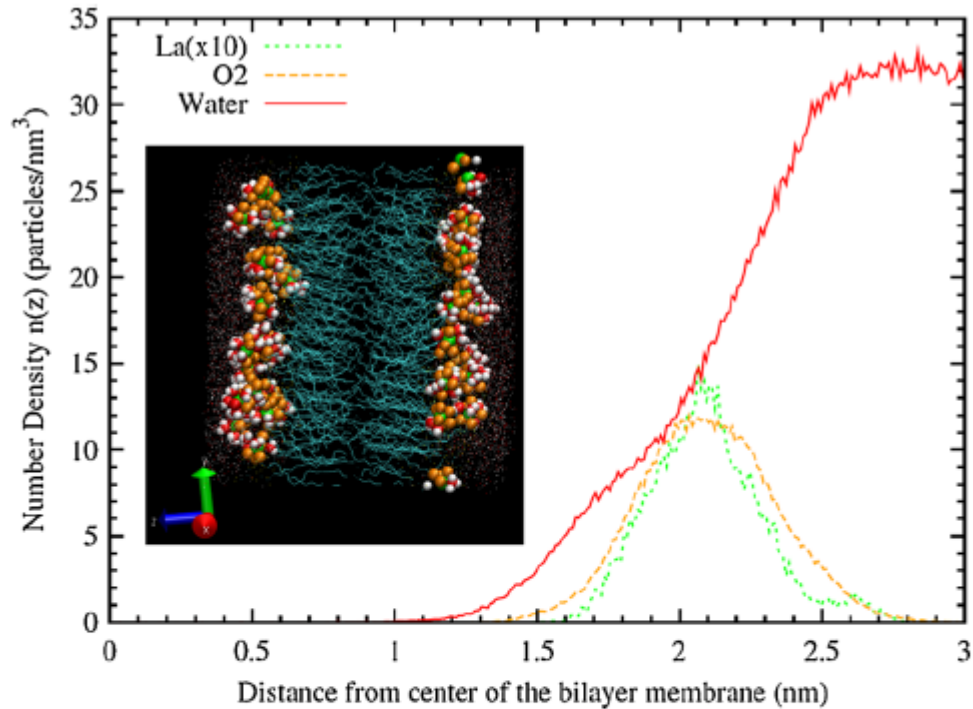


FIG. 3 (color online). Average density profile of different species as a function of the z coordinate (perpendicular to the membrane) obtained from MD simulations. Solid line: water density (molecules/nm³), dashed line: oxygen atoms of O2 type from PS⁻ molecules (atoms/nm³), dotted line: number density of La³⁺ cations (ions/nm³) multiplied by a factor 10 for clarity. Inset: snapshot from MD simulations showing the oxygen lipids, La ions and hydration water as spheres and the other atoms as lines.